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# Correlation of Effects of Inhaled Versus Intratracheally Injected Metals on Susceptibility to Respiratory Infection in Mice

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JOURNAL ARTICLE

# Correlation of Effects of Inhaled Versus Intratracheally Injected Metals on Susceptibility to Respiratory Infection in Mice[1-4]

GARY E. HATCH, RALPH SLADE, ELIZABETH BOYKIN, PING C. HU, FREDERICK J. MILLER, and DONALD E. GARDNER

SUMMARY Effects of intratracheally administered air pollutant chemicals on susceptibility of mice to infection by aerosolized bacteria were compared with effects of the same chemicals previously studied by the inhalation route. Out of 22 compounds previously studied by inhalation, the same 3 ($CdSO_4$, $CuSO_4$, and $ZnSO_4$) that caused significantly increased mortality because of infection after a 3-h exposure to 1 to 2 mg/m³ showed large (> 40% over sham injected) mortality increases when administered by intratracheal injection at lung concentrations calculated to be equivalent to those achieved during inhalation exposures (0.6 to 1.2 μg). Dose-effect curves of these compounds were similar by the 2 routes. Five of the compounds caused a moderate (20 to 30%) enhancement in the mean excess mortality at concentrations of 2 to 3 μg/animal. The remaining compounds, which showed no significant effect by inhalation, also showed no effect when given by intratracheal injection. Thus, similar effects on susceptibility to lung bacterial infection were seen whether chemicals were deposited in the lung by inhalation or by intratracheal injection.

AM REV RESPIR DIS 1981; 124:167-173

## Introduction

Mortality of experimental animals exposed to pollutants and then infected with aerosolized bacteria has been used as a sensitive indicator of lung damage caused by inhalation exposure to environmental pollutants (1-4). Photochemical oxidants and certain heavy metals (Cd, Ni, Zn, Cu) have been shown to depress host resistance when inhaled at concentrations of less than 1 mg/m³ prior to the inhalation of a bacterial aerosol. Reported here are results of an effort to apply intratracheal injection techniques to a mouse bacterial infectivity system for the purpose of developing a screening test of toxicity. Such a test could be used to determine priority chemicals for larger scale inhalation studies, thereby enabling a more cost-effective evaluation of a greater number of chemicals. It would also provide a means for studying samples collected from ambient air, which would not be available in large enough quantities for inhalation studies. Methods for rapidly injecting large numbers of mice by the intratracheal route were developed along with quality assessment procedures. Chemicals that have been studied previously by inhalation were studied by intratracheal injection and the results compared. Previous studies that used intratracheal instillation often did so because of the possibility of injecting larger quantities of chemical into the lung than would normally enter by inhalation exposure. Here we have attempted to calculate the dose delivered to the lung during inhalation exposures, and to inject amounts of chemical comparable with those calculated. Several studies that evaluated the effects of 23 inhaled chemicals on susceptibility of mice to streptococcal infection were chosen for comparison (1, 4-7; Ehrlich, R., Illinois Institute of Technology Research Institute, Chicago, IL, personal communication).

## Methods

*Animals.* Pathogen-free female mice (CD-1, COBS) weighing 18 to 23 g (approximately 4 to 5 wk old), obtained from Charles River Breeding Labs, Wilmington, MA, were used in all experiments. Animal-holding facilities conformed to the standards of the American Association for Accreditation of Laboratory Animal Care.

*Chemicals.* Cadmium sulfate was obtained from Allied Chemical, Morristown, NJ. Aluminum ammonium sulfate, ammonium, potassium, and sodium nitrates, and zinc sulfate were obtained from Sigma Chemical Co., St. Louis, MO. Ammonium sulfate, calcium nitrate, and ferric sulfate were purchased from Mallinkrodt Inc., Paris, KY, ferric oxide and powdered carbon from Pressure Chemical Co., Pittsburgh, PA., zinc ammonium sulfate from ICN Pharmaceuticals, Plainview, NY; all other compounds used for intratracheal injections were obtained from Fisher Chemical, Fair Lawn, NJ. Carrier free [$^{125}$I]sodium iodide (specific activity ~ 17 Ci/mg), was purchased from New England Nuclear, Boston, MA.

*Preparation of [$^{125}$I]proteins.* Experiments using [$^{125}$I]proteins for injection were conducted to determine whether all of the injected fluid reached the lung under the established conditions. Labeled protein was used because it would presumably not be absorbed into the blood during the period in which the mice were killed and organs removed. Because of its availability for other experiments, labeled rabbit immunoglobulin was used. This was purified from serum by DEAE cellulose column chromatography and ammonium sulfate precipitation (8). Iodination followed the method of McConahey and Dixon (9). Immediately after the iodination, the sample was eluted through a Sephadex G-50 column to separate the unbound iodine. The protein fractions were pooled and dialyzed extensively with several changes of phosphate buffered

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[2] Presented in part at the 1981 Society of Toxicology Conference, San Diego, California.

[3] Requests for reprints should be addressed to Dr. Gary E. Hatch, MD-82, U.S. EPA, Research Triangle Park, NC 27711.

[4] This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

saline at 4° C. More than 95% of the radioactivity was trichloroacetic acid precipitable.

*Intratracheal injection procedure.* Mice were anesthetized using a 1.5-min exposure set at 5% halothane (Halocarbon Laboratories, Inc., Hackensack, NJ) administered by a Frazer-Sweatman vaporizing anesthesia apparatus (Cyprane North America Inc., Tonawanda, NY). Exposure to halothane was carried out in a 2.5-L plexiglass box (Ealing Corp., So. Natick, MA). The flow rate of air through the box was 2.0 L/min. The above procedure was adequate to rapidly anesthetize mice to a degree that noticeably depressed respiration.

The needle used for the intratracheal injections was a 24-gauge intragastric feeding needle for infant animals sold by Popper & Son, Inc., New York, NY, with a 1.25-mm diameter ball on the tip. A 10° bend was made about 0.5 mm from the tip of the needle. Glass luer slip syringes of 0.25 ml volume marked in 10-μl increments (Becton-Dickinson, Rutherford, NJ) were used throughout. The usual injection volume of 10 μl per animal was expelled by a careful twisting motion of the plunger on the syringe. When suspensions were being injected, the syringe was refilled often with freshly mixed material.

Mice were hung vertically by the front upper incisors on a small wire attached to a heavy vertical support (figure 1). With the mouse facing the researcher, the tongue was grasped with forceps and pulled to the side and down. At the same time, a needle with a syringe attached was passed into the mouth and pressed ventrally behind the tongue until it could be felt to pass through the epiglottis and into the trachea. The techniques of feeling the epiglottis and tracheal rings can be gained with practice using dead mice on which the trachea has been exposed. Equipment for seeing into the mouth of the animal did not prove to be necessary or helpful. If there were doubts as to whether the needle was actually in the trachea, the mouse was excluded from the experiment. Fewer than 5% of the animals used had to be eliminated from the experiments because of a questionable injection, and more than 98% of the animals included in experiments for quality assessment showed evidence that the needle insertion was in the trachea.

Fig. 1. Photograph of the method used for intratracheal injection of mice.

*Injection of chemicals and infectious challenge.* Chemicals were dissolved or suspended in sterile 0.9% saline for injection (Abbott Laboratories, North Chicago, IL). As many as 5 treatment groups, composed of 30 randomly selected mice per group, were injected by a single technician in a given experiment. One group was always injected with saline to serve as a control. One mouse from each group was injected in sequence to randomize possible time dependent effects. After injection, anesthetized mice were put immediately into individual compartments of an open-mesh stainless steel cage. The time required to inject all animals ranged from 1 to 1.5 h. One hour after injection of the last mouse, cages were placed in a stainless steel inhalation chamber and exposed for 20 min to a viable aerosol of *Streptococcus pyogenes* (Group C) containing approximately $10^9$ colony-forming units/ml as described previously (10). This technique causes deposition of 400 to 4,000 colony-forming units of bacteria per mouse lung. It has been shown that the mortality observed in control mice is not correlated with bacterial deposition over this range (3, 7). After bacterial exposure, the animals were separated into their appropriate groups and the incidence of mortality observed over a 15-day period.

*Statistics.* Per cent mortality caused by infection was calculated for each treatment group. Excess mortality was derived for each experiment by subtracting the per cent mortality in saline-injected control animals from that seen in groups injected with chemical. The one-tailed *t* test was used to evaluate the significance of the mortality increases caused by injected chemicals. This test was judged to be more suitable than the chi-square analysis of pooled mortality data used in previously reported inhalation studies because of the intraexperimental differences that exist in the per cent mortality in control animals not exposed to pollutant. At least 3 experimental replications were made at each dosage of interest in intratracheal injection studies.

*Dosimetry of inhaled compounds in the lung.* In order to accomplish the major goal of this study, to compare inhalation with intratracheal injection exposure to chemicals, it was necessary to ensure that the dosage of chemical in the lung in each case was

TABLE 1

LACK OF EFFECT OF 5% HALOTHANE ANESTHESIA AND NEEDLE INSERTION INTO THE TRACHEA ON SUSCEPTIBILITY OF MICE TO INFECTION BY AEROSOLIZED BACTERIA*

| Treatment | n | Mortality (% ± SE) |
|---|---|---|
| Normal anesthesia, 1.5 min | 90 | 5.6 ± 2.2 |
| Prolonged anesthesia, 3.5 min | 60 | 3.3 ± 3.3 |
| Normal anesthesia plus needle insertion | 90 | 5.6 ± 2.2 |

* All animals were subjected to aerosolized *Streptococcus pyogenes* within 1 h of the treatments shown. Mean per cent mortality and standard errors are given for the number of animals shown.

similar. An estimate of the amount of chemical deposited in the lungs (μg/exposure) was obtained by multiplying the following 4 factors: (*1*) air concentration of chemical to which mice were exposed (μg/m³), (*2*) time of exposure (hours), (*3*) hourly volume breathed per mouse (m³/h), and (*4*) fraction of inhaled dose retained in the lungs immediately after exposure. Air concentrations of chemical, which were expressed in terms of either the metal or the anion in the inhalation studies, were converted to the concentration of total anhydrous salt. The published inhalation studies used either a 2- or 3-h exposure time. The average weight of mice used in this study (21.5 ± 2.55 SD g, n = 56) served as a basis for calculation of the hourly volume breathed per mouse of 0.00103 m³ using the scaling factors of Stahl (11). Per cent retention of the inhaled compounds was estimated from previous studies from our laboratory, which related air concentrations of nickel and cadmium chloride, and manganese oxide aerosols to lung concentrations after inhalation exposure (4, 5, 12). All 3 metals showed linear deposition in the lung with increasing air concentrations, and between 9 and 15 μg of metal per gram dry lung was detected after a 2-h exposure of mice to 1 mg/m³. For converting μg/g dry lung to μg/mouse, experimentally derived factors of 0.219 g dry lung/g wet lung and 0.142 g wet lung/22 g mouse were used. The percentage of inhaled metal retained in the lung immediately after inhalation exposure was calculated by dividing the actual amount of metal detected in the lungs after exposure by the amount of chemical that would have entered the lung based on physiologic parameters of the mice and air concentrations of the metal. Calculations of percentage deposition yielded 18.8, 20.2, and 26.04% of inhaled cadmium, nickel, and manganese, respectively. Although these values seemed high in comparison with the 10% or lower deposition values of Raabe and co-workers (13) reported for hamsters and rats, our calculations of cadmium deposition in rats derived from data from the same studies (4) showed 9.6% of respirable aerosol deposited agreeable to the study of Raabe and co-workers. Therefore, mice appeared to have a higher percentage of deposition than rats in the inhalation studies. For the present study it was assumed that 20% of the amount inhaled was deposited in the lungs.

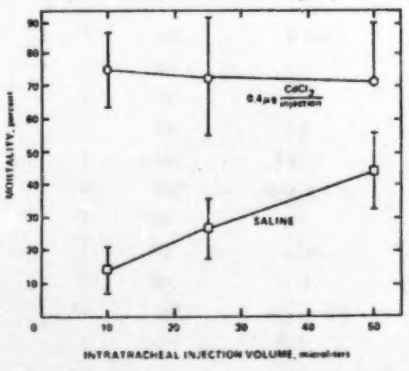


Fig. 2. Effect of intratracheal injection volume on mortality of mice because of infection by aerosolized *Streptococcus pyogenes*. Each point represents the mean ± SE of 3 experiments (30 animals each) in which different volumes of saline (□) or saline containing cadmium chloride (○) such that a fixed 0.4-μg dose was delivered to the lungs at each injection volume. The remainder of the present study used the 10-μl injection volume.

## Results

*Effects of needle insertion, anesthesia, and saline injection on bacterial infectivity.* Anesthesia induced by 5% halothane was found to inhibit the gagging reflex of the animals more effectively than did pentobarbital. It also showed shorter times for induction (1.5 min) and recovery (1.5 to 3.0 min). In addition, a 100% survival rate of intratracheally injected animals was consistently possible with the halothane anesthesia, whereas the survival rate of animals anesthetized with adequate dosages of pentobarbital was lower and inconsistent. It is shown in table 1 that only a slight statistically nonsignificant enhancement of mortality may result from halothane anesthesia and insertion of the needle into the trachea. In figure 2, the effect of injection of different volumes of saline into the lung with and without a fixed dose per lung of cadmium chloride is shown. The increased mortality caused by cadmium appeared to be the same regardless of the injection volume. Saline injection without cadmium, in 10- to 50-μl volumes, had a slight to moderate volume-dependent enhancing effect on infectivity. The injection volume chosen for the remainder of the study was 10 μl per mouse. This choice was made based on the desire to minimize the per cent mortality in the saline-injected animals for statistical reasons, and on the finding that the injection volume did not appear to significantly affect the toxicity of an injected metal.

*Disposition of intratracheally injected chemicals.* The [$^{125}$I]proteins were injected intratracheally to determine whether all of the injected fluid reached the airways and lung. Intratracheal injections of [$^{125}$I]gamma globulin into the lungs of mice was followed within 2 h by removal of the lungs and trachea. Radioactivity counts of the tissues were used to calculate the volumes injected and their distribution. Results shown in table 2 indicated that approximately 70% of

TABLE 2

DISTRIBUTION OF INTRATRACHEALLY INJECTED SALINE CONTAINING [$^{125}$I]PROTEIN

| Condition | Volume (μl)* | Distribution of Injected Protein (%) |
|---|---|---|
| 1. Total expelled from syringe directly into vials | 10.08 ± 1.42 μl/vial | — |
| 2. Total injected intratracheally | | |
| whole animal | 9.95 ± 2.44† μl/mouse | — |
| lungs and trachea | 6.97 ± 2.92 μl/mouse | 70.1 |
| head | 0.73 ± 0.31 μl/mouse | 7.3 |
| carcass (remaining body) | 2.59 ± 1.96 μl/mouse | 26.0 |

* Mean ± SD, n = 30 mice per condition.
† All radioactivity counts were converted to microliters of volume of injected protein solution. Specific activity of the solution was determined by weighing vials in Condition 1.

the intratracheally injected material remained in the lung and trachea after injection. Only about 7% of the injected material stayed in the head. The other 26% appeared in the carcass, and essentially all of this fraction was found to be in the stomach. Variability in the amounts injected was due both to pipetting error and to variability in the percentage of the material that was swallowed.

*Effects of intratracheally injected compounds on susceptibility to infection.* Twenty-two compounds were injected intratracheally in concentrations that approximated those that would have been reached in previously reported inhalation studies. In table 3, the amount of each chemical (expressed in terms of the total anhydrous salt) that would have been present in the lungs of mice after intratracheal and inhalation exposures is shown. Lung dosages of intratracheally injected compounds reflect the 70% deposition value determined from radiolabeled protein injections (table 2), and lung dosages in inhalation studies were calculated from air concentrations and exposure times, as described in *Methods*. With intratracheal exposures, mean excess mortality and standard errors represented 3 to 5 replicates of an experiment, each replicate composed of 30 mice. Inhalation studies usually used fewer replications of a given dose with linear fitting of data from several dosages when an enhancement in mortality was seen. Chemicals that did not

TABLE 3

COMPARISON OF INTRATRACHEAL INJECTION AND INHALATION OF CHEMICALS ON SUSCEPTIBILITY TO STREPTOCOCCAL INFECTION IN MICE

| | Intratracheal Injection | | | Inhalation | | | |
|---|---|---|---|---|---|---|---|
| Chemical | Estimated Lung Dosage, (μg/mouse) | Excess Mortality (%)* | n | Estimated Lung Dosage (μg/mouse) (mg/m³ inhaled) | Excess Mortality (%) | n | Reference |
| Aluminum ammonium sulfate $ALNH_4(SO_4)_2$ | 2.10 | 20.9 ± 0.9† | 90 | 2.90 (4.69) | 15.3 ± 0.8 | 118 | ‖ |
| Aluminum sulfate $AL_2(SO_4)_3$ | 2.49 | 4.2 ± 7.1 | 120 | 2.96 (4.85) | 23§ | 48 | 1 |
| Ammonium nitrate $NH_4NO_3$ | 2.10 | −6.4 ± 8.7 | 120 | 3.60 (5.81) | −6.3 | 48 | ‖ |
| Ammonium bisulfate $NH_4HSO_4$ | 2.51 | 41.1 ± 4.0† | 90 | 4.80 (8.02) | 6.7 | 48 | ‖ |
| Ammonium sulfate $(NH_4)_2SO_4$ | 2.80 | −6.7 ± 3.3 | 120 | 4.38 (7.07) | 2.2 | 110 | 5 |
| Cadmium chloride $CdCl_2$ | 0.46 | 39.4 ± 8.7† | 180 | 0.44 (1.0, 2 h) | 49.0§ | > 500 | 4 |
| Cadmium sulfate $CdSO_4$ | 3.89 | 60.0 ± 20.0† | 60 | 2.45 (3.95) | 76.7 | 46 | ‖ |
| | 1.30 | 67.5 ± 9.5† | 120 | 0.86 (1.39) | 80.9 | 48 | |
| | 0.39 | 43.3 ± 6.5† | 120 | 0.60 (0.96) | 20.8 | 48 | |
| | 0.13 | −1.7 ± 5.0 | 60 | 0.29 (0.48) | | | |
| Calcium nitrate $Ca(NO_3)_2$ | 2.10 | −1.1 ± 3.6 | 90 | 2.29 (3.70) | −5.7 | 48 | 1 |
| Carbon black | 3.50 | 11.9 ± 8.1 | 90 | 2.29 (3.70) | < 8.0 | 140 | 7 |
| Copper sulfate $CuSO_4$ | 2.10 | 62.5 ± 10.3† | 120 | 2.19 (3.54) | 91.7 | 48 | ‖ |
| | 0.70 | 37.1 ± 10.1† | 120 | 1.10 (1.78) | 68.8 | 48 | |
| | 0.21 | 5.0 ± 4.8 | 120 | 0.89 (1.44) | 27.1 | 48 | |
| | | | | 0.70 (1.13) | 8.3 | 48 | |
| Ferric ammonium sulfate $FeNH_4(SO_4)_2$ | 4.12 | 39.1 ± 4.1† | 150 | 3.03 (4.90) | 13.3 | 48 | 1 |
| | 1.37 | 13.6 ± 5.3 | 90 | 3.03 | | | |
| Ferric oxide $Fe_2O_3$ | 3.50 | 13.0 ± 9.1 | 90 | 1.03 (2.5, 2 h) | < 2.0 | 140 | 7 |
| Ferric sulfate $Fe_2(SO_4)_3$ | 2.10 | 12.2 ± 9.5 | 90 | 3.75 (6.04) | 1.3 | 48 | 1 |
| Lead nitrate $Pb(NO_3)_2$ | 5.61 | 9.2 ± 11.0 | 120 | 3.30 (5.34) | 0.8 | 48 | 1 |
| Magnesium sulfate $MgSO_4$ | 10.5 | 5.6 ± 12.1 | 90 | 1.93 (3.12) | 9.2 | 95 | 1 |
| Manganous chloride $MnCl_2$ | 3.21 | 5.4 ± 10.4 | 150 | 3.1 (5.0) | 18§ | 240 | 7 |
| Nickel chloride $NiCl_2$ | 3.08 | −9.1 ± 6.0 | 150 | 0.69 (1.11, 2 h) | 13.7 ± 10.6 | 160 | 6 |
| Potassium nitrate $KNO_3$ | 2.10 | −8.9 ± 1.1 | 90 | 4.34 (7.00) | −4.2 | 48 | ‖ |
| Sodium sulfate $Na_2SO_4$ | 2.13 | 8.17 ± 4.08 | 120 | 5.92 (4.0) | −10.7 | 48 | ‖ |
| Sodium nitrate $NaNO_3$ | 2.10 | 2.5 ± 12.9 | 120 | 2.63 (4.25) | 0.1 | 48 | ‖ |
| Vanadyl sulfate $VOSO_4$ | 2.10 | 22.2 ± 10.9 | 90 | 1.19 (1.93, 2 h) | 5.5 ± 1.81 | 560 | 4‡ |
| Zinc ammonium sulfate $Zn(NH_4)_2(SO_4)_2$ | 3.19 | 25.0 ± 11.0† | 120 | 2.11 (3.40) | 50§ | 760 | 5 |
| | 1.06 | −2.2 ± 19.2 | 90 | 1.50 (2.42) | 25§ | | |
| Zinc sulfate $ZnSO_4$ | 2.10 | 75.0 ± 7.0† | 120 | 2.36 (3.81) | 60§ | 1,600 | 5 |
| | 0.70 | 19.2 ± 4.4† | 120 | 1.40 (2.27) | 20§ | | |
| | 0.21 | 2.5 ± 1.1 | 120 | | | | |

* Mean ± SE, n = number of mice studied.
† $p < 0.05$, the $t$ test by difference greater than zero.
‡ Inhalation exposure to $VOSO_4$ was performed according to methods of Reference 4 but first reported here. Aerosol mass median aerodynamic diameter was between 0.56 and 1.13 μm.
§ Excess mortality values taken from a fitted curve of dose effect data.
‖ Ehrlich, R. Illinois Institute of Technology Research Institute, Chicago, IL; personal communications.

enhance mortality in inhalation studies are listed in the table along with the highest concentration at which they were tested and the number of animals included in the test.

Three of the 22 chemicals injected (cadmium sulfate, copper sulfate, and zinc sulfate) caused significant ($p \leq 0.05$) enhancements in mortality at lung concentrations of 1 μg/animal or less. Cadmium chloride was not included in the correlation because of its similarity to cadmium sulfate. Two chemicals (aluminum ammonium sulfate and ammonium bisulfate) caused significantly enhanced mortality (20.9% and 41.1%) at 2.10 and 2.51 μg lung concentrations, respectively. Ferric ammonium sulfate significantly increased mortality (39.1%, $p < 0.05$) at a higher dosage of 4.12 μg and appeared to have some effect at dosage of 1.37 μg (13.6%, $p = 0.06$). Two chemicals that caused more variable elevations in mortality were zinc ammonium sulfate (25%, $p = 0.06$) at a dosage of 2.16 μg and vanadyl sulfate (22%, $p = 0.09$) at a dosage of 2.10 μg. The remaining 13 chemicals caused no significant increases in mortality at lung concentrations of 2 μg or higher. The dose-effect relationships seen with the more toxic chemicals and a comparison between results of inhalation and intratracheal injection are shown in figure 3. The abcissa of both the inhalation and intratracheal injection portions of the figure are aligned so that results expressed in terms of inhaled air concentrations can be compared according to the amount of chemical delivered to the lungs. Because data from some of the inhalation studies were plotted arithmetically, the original numerical data were obtained and plotted logarithmically. Comparison of dose-effect curves of inhalation and intratracheal injection routes indicated general agreement in the results. Graphically estimated 20% effective dose $ED_{20}$ values were as follows for intratracheal and inhalation studies, respectively: $CuSO_4$, 0.35 and 0.90 μg/mouse; $CdSO_4$, 0.24 and 0.38 μg/mouse; $ZnSO_4$, 0.75 and 2.3 μg/mouse.

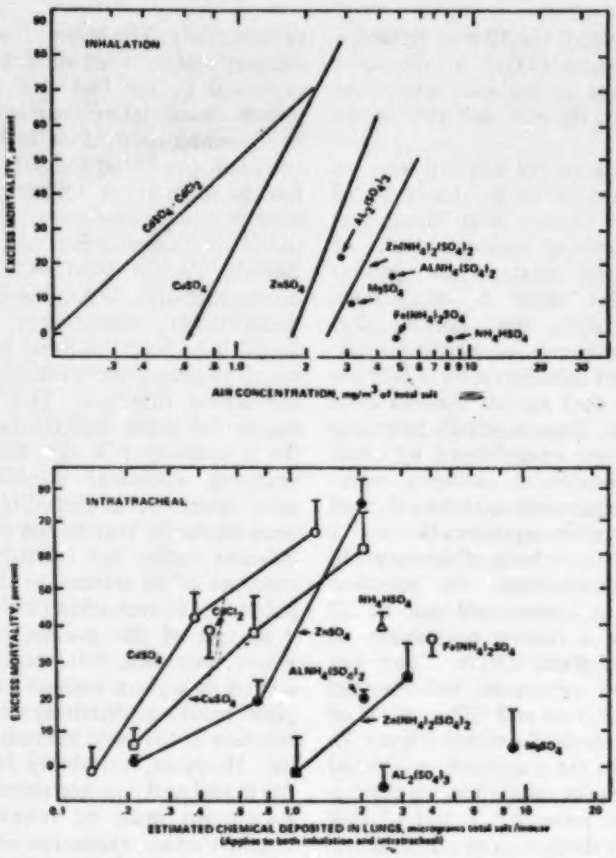
INHALATION
EXCESS MORTALITY, percent
AIR CONCENTRATION, mg/m³ of total salt
INTRATRACHEAL
EXCESS MORTALITY, percent
ESTIMATED CHEMICAL DEPOSITED IN LUNGS, micrograms total salt / mouse
(Applies to both inhalation and intratracheal)

Fig. 3. Comparison of inhaled versus intratracheally instilled chemicals on susceptibility of mice to aerosol infection by *Streptococcus pyogenes*. The upper portion of the figure shows dose-effect curves of inhaled metal sulfates, which were replotted from Ehrlich (1, 9) and Gardner and associates (4) so that concentrations were expressed in terms of total anhydrous compound rather than sulfate or nitrate. The lower portion shows dose-effect curves of selected compounds studied by intratracheal injection. The lower abcissa, expressed in micrograms per animal, is aligned with the upper figure, according to procedures outlined in *Methods*, so that a comparison of results of equivalent lung concentrations of compounds can be made directly from the figure.

## Discussion

Lung infection of mice by bacterial aerosol, a technique that has been used previously as an endpoint for the inhalation toxicity of environmental pollutants, has here been studied using the intratracheal injection route of exposure to the pollutant compounds. The purpose of this study was to compare results obtained using intratracheal injection with data from inhalation studies of the same compounds. Similarity in the effects seen by the 2 routes of exposure was taken as evidence that intratracheal injection procedures have some meaning when used with this toxicologic endpoint. Use of intratracheal injection would simplify the study of compounds for which inhalation studies are difficult, and could provide a prescreening for determining priority chemicals for inhalation studies. It would also enable evaluation of compounds that are available only in small quantities.

Because previous infectivity studies used mice principally, methods were perfected for rapid intratracheal injection of this animal. Characteristics of the intratracheal injection technique were explored. Effects of brief halothane anesthesia, needle insertion, and injection of small volumes of saline (10 μl) were found to be slight, and did not appear to affect the toxicity of the injected compound as measured by susceptibility to infection after inhalation of an aerosol of *Streptococcus pyogenes*. Injection of [$^{125}$I]proteins served as a quality assurance technique and showed that an average of 26% of the injected dose was swallowed; the other 70% was deposited in the lungs. Although the amount of intratracheally injected fluid that was swallowed was larger than expected, it is pertinent to note that inhalation exposure also resulted in a large amount of material being swallowed compared with what was deposited in the lung. Henderson and co-workers (14) reported that nose-only exposure of hamsters to a

$^{115}CdCl_2$ aerosol for 30 min yielded a large percentage (43%) of the total animal dosage in the gastrointestinal tract, 20% on the pelt, and 19% in the lungs.

Application of the intratracheal injection technique to the study of 22 compounds showed that there was general agreement between results of inhalation and intratracheal injection studies. The same 3 compounds ($CdSO_4$, $CuSO_4$, and $ZnSO_4$) that caused significantly increased mortality because of infection after a 3-hr exposure to 1 to 2 mg/m³ showed large (> 40% over sham injected) mortality increases when administered by intratracheal injection at amounts calculated to be equivalent to those achieved during inhalation exposures (0.6 to 1.2 mg/lungs). On the basis of simple probability determinations, the selection of 3 distinct compounds out of 22 would have a chance probability of occurring of about 0.01%. There was also general agreement between the dose-effect curves and $ED_{20}$ values of the 3 more toxic chemicals (figure 3). The fact that the graphically estimated $ED_{20}$ values for inhalation studies appeared to be between 1.5- and 3.1-fold higher than those seen in intratracheal injection may reflect variability of the 2 techniques or uncertainty of the assumptions made in expressing the lung dosage concentration in inhalation exposure studies. This discrepancy might also be caused by a difference in the distribution of chemical within the lungs. Inhalation exposure would probably result in a wider distribution within the lung, which may have a diluting effect on the toxicity of the chemical.

Five of the chemicals appeared to have an intermediate effect when given by intratracheal injection, e.g., they showed mortality increments significant at $p < 0.1$ at concentrations higher than 2 μg/lungs. Within this group, zinc ammonium sulfate was also reported to exert moderate effects by inhalation (figure 3 and table 3). Ammonium bisulfate, ferric ammonium sulfate, and aluminum ammonium sulfate showed moderate effects by intratracheal injection, but they were not reported to have a significant effect in the inhalation studies of Ehrlich (1). Conversely, magnesium sulfate and aluminum sulfate were reported to have moderate effects by inhalation but showed no significant effects in the present study. The lesser effect of ammonium sulfate when inhaled might be explained by the fact that ammonia present in inhalation chambers caused by decomposition of animal urine is sufficient to convert ammonium bisulfate to ammonium sulfate such that animals were actually being exposed to the latter compound (Cannon, WC. Battelle Pacific Northwest Laboratories, Richland, WA., personal communication). Ammonium bisulfate would have been less likely to be converted to the sulfate when given by intratracheal injection. The probable reason for other differences between the 2 techniques is the difficulty in assigning statistical significance to small increments in mortality. This has been especially true in the case of inhalation studies that often use only 1 exposure of 48 animals to the highest pollutant concentration (table 3).

Results of the present study suggested, therefore, that intratracheal injection of a given amount of chemical yields results qualitatively similar to inhalation of the same amount of chemical. However, variability inherent in the model and possible chemical transformations must be taken into account. Further validation of the technique is required especially for compounds that differ in their physicochemical properties from those studied here. These results were somewhat surprising in view of the fact that the distribution of intratracheally instilled particles, especially in smaller volumes, is not uniform throughout the lung as in the case with inhalation exposure (15). Instillation and inhalation of the chemicals studied here yielded similar results, apparently in spite of these differences in distribution. Because exposure to the infectious agent was by inhalation, which would have resulted in evenly distributed bacteria, it is likely that susceptibility of the whole lung to infection is related to susceptibility of the area of weakest resistance, which might be only a small portion of the total inner surface area of the airways and lung.

Reasons for the large differences between the compounds studied here in the way they alter susceptibility to infection in the lung are not well understood. To some extent these differences seem to be related to general cytotoxicity of the chemicals (16); however, no single mechanism that has been investigated can account for the sensitivity and pattern of effects seen in the *in vivo* model (2). It is therefore probable that multiple mechanisms are involved, including damage to ciliary and macrophage functions, and alteration of epithelial cells and surfactant materials. The mouse infectivity system used in this study appears to provide an overall assessment of the total ability of the animal to resist airborne acute infectious agents. Experiments with animal species other than mice, as well as other infectious agents, have also been tried with similar results (2). The question of whether data from animal models of infection are relevant to human respiratory disease can be addressed only by making comparisons with human epidemiologic data. Studies to date on cigarette smoke and nitrogen dioxide have indicated that the severity and incidence of respiratory infections in both humans and animals is associated with inhalation of these toxic materials (2, 10, 16-22).

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